Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1963, **29**, 803-811

Pathogenesis of Rabies *

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The authors have investigated the spread of fixed and street strains of rabies virus from the site of injection to the central nervous system and salivary glands in various animal species. The results indicate conclusively that rabies virus is ordinarily transmitted from the site of exposure to the central nervous system via *the peripheral nerves but that other than nerve transmission may occur in young animals, in highly susceptible species or in animals whose resistance has been altered by trauma or shock. Air-borne infection is occasionally possible. Blood-borne infection in nature is believed to be exceptional and less likely to occur in man, whose resistance to rabies is high, than in animals of species known to be highly susceptible. Evidence of nerve-borne transmission was also observed with herpes simplex virus but not with lymphocytic choriomeningitis virus or the GD7 and FA strains of mouse encephalomyelitis virus.*

Although the spread of rabies virus from the central nervous system of infected animals to the peripheral nerves was documented at an early date, the pathway of virus from the site of exposure to the brain has remained a controversial matter at least since the days of Pasteur (1889). As is evident from the reviews by Habel (1941), Webster (1942), Hutyra, Marek & Manninger (1946), Lépine (1948) and Burnet (1960), some believed that rabies virus passes from the infected wound *via* the peripheral nerves, others that infection is blood-borne; Pasteur (1889) and Roux (1889) entertained the possibility of infection by both routes. This study re-examines the question of the route of spread within the body of the infected host.

METHODS AND PROCEDURES

Experimental animals

Animals were selected by random sampling or by stratified random sampling according to weight and sex. Unless otherwise specified, they consisted of mice of the Albany standard or Swiss strains weighing 10-14 g; guinea-pigs, rats, and hamsters of Albany strains weighing 300-400 g, 60-100 g, and 40-60 g respectively; yearling silver foxes reared in captivity and obtained from the New York State Conservation Department; and dogs six weeks old purchased locally. The dams of all animals were known not to have been previously vaccinated against rabies. Test animals were observed daily for not less than 30 days after inoculation of virus; dogs and foxes were observed for two and three months respectively. Animals dying within the first three days after inoculation were excluded; those in critical experiments and the rare animal in all experiments dying without signs suggestive of rabies were examined by the fluorescent antibody test or mouse inoculation, or both.

Virus

The CVS strain of fixed rabies virus, pools 4 and 5, was obtained from the National Institutes of Health; pools 4 and 5 consisted of 20% infective mouse-brain suspensions and had median titres of $10^{6.7}$ and $10^{6.9}$ respectively when titrated in mice with 0.03 ml of inoculum by the intracerebral route. Infective salivary gland suspensions served as source of "street" [4] virus; strain 61-797F was isolated from

* This investigation was supported by the World Health Organization and by a United States Public Health Service Research Grant (No. 3982).

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[4] The term "street" virus, widely used to denote isolates largely unaltered by laboratory manipulation, is questioned since most isolates now originate from rural areas and differences between "street" and "fixed" strains seem less important than was previously believed.

a rabid fox obtained from Orleans County, New York, in 1961 and strain 61-797D from a dog inoculated intracerebrally with strain 61-797F. Modified live rabies vaccine of low-egg-passage, Flury type (Koprowski & Cox, 1948), was purchased commercially. Infective mouse-brain suspensions of herpes simplex and lymphocytic choriomeningitis viruses, and of the GD7, FA and 4727 strains of mouse encephalomyelitis virus were from stocks used at this laboratory. Virus preparations were stored at −70°C, with the exception of mouse encephalomyelitis viruses stored in glycerol at 4°C. Saline broth solution with or without 2%-10% normal horse serum served as diluent.

Procedures

Sucklings were etherized. Other animals undergoing surgical procedures were anaesthetized with sodium pentobarbital administered intraperitoneally or intravenously. By using the hip joint as reference, a portion of the sciatic nerve was removed through an incision 5-35 mm long in the skin and muscle parallel and caudal to the upper end of the femur. Approximately 1 cm was removed from a small mouse and as much as 5 cm from larger animals. Similarly, at least 1 cm of saphenous nerve was removed through an incision on the medial surface of the thigh; in suckling mice the accompanying saphenous vein and artery were also removed. Animals with portions of both sciatic and saphenous nerves removed are referred to as " neurectomized ". Virus was inoculated in the foot-pad of the operated limb two or more days, usually five to seven, after surgery. Virus isolations were made by inoculating intracerebrally eight to ten mice per specimen with 10%-20% tissue suspensions; five to ten mice per dilution were used in virus titrations unless otherwise specified. The volume of inoculum in all experiments was 0.03 ml unless otherwise mentioned.

The statistical significance of observed differences in proportions reacting was tested in accordance with what Fisher (1934) called " the exact treatment of two-by-two tables." This is based upon a critical probability (P) that Thompson (1934) expressed in terms of his four-variable ψ-functions. The values of the critical probabilities (P) were obtained by use of Thompson's (1962) electronic computer programmes for the IBM 650 and 1620 machines. The median-effective dilution (MED) was estimated according to the method of Thompson (1947).

EXPERIMENTS

Effect of removal of nerve segments

Since it has been demonstrated that substances interfering with nerve function exert a sparing effect in rabies (Kaplan et al., 1962; Dean et al., 1963; Wiktor & Koprowski, 1963), an attempt was first made with 25-30-g mice to determine the influence of removal of segments of either the sciatic or saphenous nerve, or both, upon the outcome of infection in animals injected in the foot-pad. Four groups containing 32-40 mice each were inoculated in the foot-pad of the right hind leg with 0.02 ml of CVS virus, pool 4. One group served as controls; mice in the other three groups had had portions of the sciatic or of the saphenous nerve or of both removed from the right hind leg two or three days before injection of virus. Whereas mortality in the controls was 38 of 40 (95%), none of the 32 and 40 mice respectively on which combined sciatic and saphenous or just sciatic neurectomies were performed died, and only 24 of 34 (70.6%) mice with saphenous neurectomies succumbed (Table 1). Essentially similar results were obtained when guinea-pigs were injected in the foot-pad with 0.25 ml of a 15% suspension of CVS virus, pool 5. Mortality in the controls was 6 of 10 (60%) as compared with none of 10 in neurectomized guinea-pigs and 1 of 19 (5.3%) and 3 of 10 (30%) respectively in guinea-pigs from which portions of the sciatic or saphenous nerves only were removed (Experiment 1, Table 1).

A sparing effect was also noted in neurectomized mice when groups of 20-30 animals were inoculated in the foot-pad with 10% salivary gland suspensions of strains 61-797F and 61-797D of rabies street virus or low-egg-passage modified live-virus vaccine (Flury) containing 33.3% tissue (Table 1); the titres were $10^{6.4}$, $10^{4.5}$ and $10^{4.5}$ respectively. Mortality in the controls was 100%, 65% and 20% as compared with 5%, 5% and 0 in neurectomized animals. One neurectomized animal in each experiment with street virus succumbed to rabies, a phenomenon also seen occasionally in mice inoculated with CVS virus. Observations were subsequently extended to additional guinea-pigs and to other species, including rats and hamsters inoculated with CVS virus, pool 5, and foxes and dogs inoculated with 0.25 ml of a 1/100 suspension of street virus strain 61-797F. The sparing effect was noted in all species but was less marked in hamsters (Table 1).

Throughout these experiments, the neurectomized mice occasionally succumbing to rabies appeared to

TABLE 1

SPARING EFFECT OF REMOVING PORTIONS OF SCIATIC AND/OR SAPHENOUS NERVES ON OUTCOME OF INFECTION IN ANIMALS INOCULATED IN THE FOOT-PAD WITH RABIES VIRUS [a]

Neurectomy	Mice				Guinea-pigs		Rats	Hamsters	Foxes	Dogs
	CVS strain, pool 4	Strain 61-797F	Strain 61-797D	LEP Flury strain	CVS strain, pool 5		CVS strain, pool 5	CVS strain, pool 5	Strain 61-797F	Strain 61-797F
					Exp. 1	Exp. 2				
Sciatic nerve only	0/40(HS)	—	—	—	1/19(HS)	—	—	—	—	—
Saphenous nerve only	24/34	—	—	—	3/10	—	—	—	—	—
Sciatic and saphenous nerves	0/32(HS)	1/20(HS)	1/20(HS)	0/29 (S)	0/10 (S)	0/39(HS)	0/10(HS)	6/9	0/2	0/4
Controls	38/40	20/20	13/20	6/30	6/10	33/38	10/10	10/10	2/2	2/2

[a] Results are expressed as the number of animals dying of rabies over the number inoculated. S indicates that the difference from the controls is statistically significant; HS that it is highly so.

be among the smaller and presumably younger animals. To test this hypothesis, 15 neurectomized and 15 non-neurectomized mice in groups of progressively varying ages, from 13 days to 42 or more days, were given injections in the foot-pad of a 15% suspension of CVS virus, pool 5. The dosage was adjusted according to weight, with mice of 5-6 g, 10-12 g and 20-24 g receiving 0.01 ml, 0.02 ml and 0.04 ml respectively. The results (Table 2) indicate that, among neurectomized mice, those of the 13-day-old group are the most susceptible. All neurectomized mice in the two older groups survived, but mortality was 13 of 15 (86.7%) in the 13-day-old group; all control animals died. The observed median survival time was 7.0 days in 13-day-old neurectomized mice and 6.0 days for control mice of similar age and weight.

Like, though less sharply contrasting, results were obtained when groups of 5-15 neurectomized and non-neurectomized rats 19, 28 and 40-43 days old were given foot-pad injections of CVS virus, pool 5. The dosage was roughly adjusted for weight: 20-23-g, 48-65-g and 108-132-g rats respectively receiving 0.03 ml, 0.06 ml and 0.125 ml of a 15% tissue suspension. All control rats in each age class died as did 9 of 15 (60.0%) 19-day-old neurectomized rats and 2 of 6 (33.3%) 28-day-old neurectomized animals, whereas all 9 of the 40-43-day-old neurectomized rats survived (Table 2).

Koprowski & Cox (1948) showed that hamsters were extremely susceptible to rabies. To compare the susceptibility of mice and hamsters of like age, groups of eight and three animals, weighing respectively 10-12 g and 40-60 g, were inoculated in

TABLE 2

EFFECT OF AGE AND DOSE ON OUTCOME OF INFECTION IN ANIMALS INOCULATED IN THE FOOT-PAD WITH THE CVS STRAIN, POOL 5, OF RABIES VIRUS [a]

Mice							Rats						
Age (days)	Weight (g)	Dose (ml)	Neurectomized: mortality		Controls: mortality		Age (days)	Weight (g)	Dose (ml)	Neurectomized: mortality		Controls: mortality	
			No.	%	No.	%				No.	%	No.	%
13	5-6	0.01	13/15	86.7	15/15	100.0	19	20-23	0.05	9/15	60.0	6/6	100.0
21-28	10-12	0.02	0/15(HS)	0.0	15/15	100.0	28	48-65	0.06	2/6	33.3	5/5	100.0
42-56	20-24	0.04	0/15(HS)	0.0	15/15	100.0	40-43	108-132	0.125	0/9 (HS)	0.0	5/5	100.0

[a] Results are expressed as the number of animals dying of rabies over the number inoculated. HS indicates that the difference from the controls is statistically highly significant.

the foot-pad with a series of increasing fourfold dilutions of CVS virus, pool 5, starting with a 15% tissue suspension. The median-effective dose for hamsters was approximately 1/16 that for mice even though the body-weight was at least three to five times as great. Further study of hamsters weighing 60-100 g inoculated in the foot-pad with CVS virus, pool 5, indicated that the sparing effect of neurectomy varied inversely with the virus dose. Whereas six of eight neurectomized hamsters inoculated with a 1: 6.7 dilution of virus died, three, four and five survived in groups of five animals given successively half the dose. All control hamsters died at each dosage level.

A sparing effect was also observed in 4.5-5.5-g neurectomized mice inoculated in the foot-pad at 15 days of age with 0.02 ml of a 20% mouse-brain suspension containing herpes simplex virus. Mortality was 3 of 22 (13.6%) in contrast to 18 of 19 (94.7%) in the controls, suggesting that herpes simplex virus, like rabies, is chiefly but perhaps not solely transmitted centripetally *via* the peripheral nerves. Removal of portions of both the saphenous and sciatic nerves, however, had no appreciable sparing effect in mice inoculated in the foot-pad with the FA and GD7 strains of mouse encephalomyelitis virus (Table 3). Similarly, no significant difference was observed in either morbidity or mortality of neurectomized and normal mice, weighing 5-7 g, inoculated in the foot-pad with 0.02 ml of a 20% infective mouse-brain suspension of lymphocytic choriomeningitis virus. All mice in both groups sickened; mortality was 6 of 16 (37.5%) in the controls and 3 of 16 (18.8%) in neurectomized mice.

TABLE 3

EFFECT OF REMOVING PORTIONS OF SCIATIC AND SAPHENOUS NERVES ON OUTCOME OF INFECTION IN MICE INOCULATED IN THE FOOT-PAD WITH HERPES, LYMPHOCYTIC CHORIOMENINGITIS AND MOUSE ENCEPHALOMYELITIS VIRUSES [a]

	Herpes virus mortality	LCM virus mortality	Mouse encephalomyelitis viruses	
			GD7 strain mortality	FA strain mortality
Neurectomized mice	3/22 (HS)	3/16	20/24	6/20
Controls	18/19	6/16	23/25	3/20

[a] Results are expressed as the number of animals dying of rabies over the number inoculated. HS indicates that the difference from the controls is statistically highly significant.

The survivors failed to grow as otherwise should be expected and had a hunched posture and extremely jumpy behaviour that has been described by Hotchin & Weigand (1961) for like circumstances.

After having confirmed that rabies travels to the central nervous system *via* the peripheral nerves, two studies in guinea-pigs were undertaken to ascertain whether virus travels by the dorsal or ventral roots, or both. In the first study four guinea-pigs in which the dorsal roots of spinal nerves from the fifth lumbar nerve caudally had been sectioned, 15 neurectomized guinea-pigs and 15 control animals were inoculated in the foot-pad with 0.25 ml of CVS virus, pool 5. Death followed in 13 of 14 (92.9%) control guinea-pigs and in 3 of 4 (75%) animals in which the dorsal roots were

TABLE 4

PATHWAYS OF VIRUS FROM SITE OF INOCULATION TO CENTRAL NERVOUS SYSTEM IN GUINEA-PIGS INOCULATED IN THE FOOT-PAD WITH THE CVS STRAIN, POOL 5, OF RABIES VIRUS [a]

Neurectomy	Experiment 1			Experiment 2		
	Mortality		Median survival time (days)	Mortality		Median survival time (days)
	No.	%		No.	%	
Dorsal roots	3/4	75.0	8.0	2/3 [b]	66.7	8.0
Ventral roots	—	—	—	2/4 [c]	50.0	7.0
Sciatic and saphenous nerves	0/15 (HS)	0.0	—	0/15 (HS)	0.0	—
Controls	13/14	92.9	6.0	10/15 [d]	66.7	7.5

[a] Results are expressed as the number of animals dying of rabies over the number inoculated. HS indicates that the difference from the controls is statistically highly significant.

[b] One additional guinea-pig died of causes other than rabies on the 15th day after inoculation.

[c] One additional guinea-pig died of causes other than rabies on the 10th day after inoculation.

[d] One additional guinea-pig became paralysed in the 10th day but survived the test period.

sectioned, thus suggesting that virus may also ascend ventral roots. All neurectomized guinea-pigs survived (Table 4).

In the second study, groups of 15 neurectomized and non-neurectomized guinea-pigs, together with three and four guinea-pigs in which portions of the dorsal and ventral roots respectively had been removed caudally from the fifth lumbar nerve had injections in the foot-pad with the same virus dosage. Mortality in the controls was 10 of 15 (66.7%), one additional guinea-pig becoming paralysed on the tenth day after injection but surviving the 30-day test period. Guinea-pigs with sciatic and saphenous nerves sectioned survived. Two of the four guinea-pigs whose dorsal roots were sectioned died of rabies, confirming the results obtained in the first study. Two of the three guinea-pigs whose ventral roots were removed also died of rabies, suggesting that virus may also traverse the dorsal roots (Table 4). The possibility of transmission of virus *via* the autonomic nervous system in guinea-pigs in which portions of the dorsal or ventral roots of spinal nerves were removed cannot be excluded.

Rate of centripetal virus transmission

To measure the rate of transmission of virus centripetally in nerve trunks, groups of 15 mice each were inoculated in the front or hind foot-pad with 0.01 ml or 0.02 ml, respectively, of CVS virus, pool 5. The inoculated extremity of all mice except the controls was amputated approximately 12 mm above the site of injection in the region of the upper humerus or femur 15 minutes or 1, 2, 4, 8, 12, or 24 hours after injection. Mortality in the controls was 13 of 15 (86.7%) given the injection in the front limb and 14 of 15 (93.3%) injected in the hind limb. Only one mouse died of rabies in those groups in which amputation was performed two hours or less after injection. Mortality from rabies was 2 of 11 (18.2%) and 6 of 14 (42.9%), respectively, in mice whose front or hind limbs were amputated four hours after injection; when amputation was delayed eight hours or more, all mice, or all but one mouse, in each group died (Experiment A, Table 5). If multiplication of virus after injection is not essential, as seems likely, the results suggest that rabies virus may be crudely estimated to travel along nerve pathways

TABLE 5

EFFECT OF AMPUTATION OF INJECTED LIMB ON OUTCOME OF INFECTION IN MICE INOCULATED IN THE FOOT-PAD WITH RABIES OR MOUSE ENCEPHALOMYELITIS VIRUS [a]

Interval between inoculation and amputation	Rabies virus (CVS strain, pool 5)				Mouse encephalomyelitis virus (strain 4727)	
	Experiment A		Experiment B		Experiment C	Experiment D
	Front limb	Hind limb	Non-neurectomized Hind limb	Neurectomized [b] Hind limb	Hind limb	Hind limb
5 minutes	—	—	—	—	8/28	—
15 minutes	1/14 (HS)	0/13 (HS)	—	—	10/35	—
30 minutes	—	—	—	—	9/36	—
1 hour	0/10 (HS)	0/15 (HS)	0/25 (HS)	0/25	9/33	20/41
2 hours	0/12 (HS)	0/14 (HS)	—	—	—	—
4 hours	2/11 (HS)	6/14 (HS)	0/18 (HS)	0/21	—	21/40
8 hours	15/15	15/15	20/23	0/19	—	—
12 hours	14/15	15/15	25/25	0/21	—	—
24 hours	14/15	14/14	—	—	—	25/39
72 hours	—	—	—	—	—	19/43 (S)
Controls	13/15	14/15	25/25	3/25	6/41	29/42

[a] Results are expressed as the number of animals dying of rabies or mouse encephalomyelitis over the number inoculated. S indicates that the difference from the controls is statistically significant; HS that it is highly so.

[b] In Experiment B neurectomy consisted in transection of the sciatic or saphenous nerves or removal of a nerve segment of not more than 2 mm at the distal end of the femur.

at a rate of 3 mm or faster per hour, approximately the rate measured for poliomyelitis virus by Bodian & Howe (1941).

To preclude the possibility of infection of severed nerves at the site of amputation by blood-borne virus, a similar experiment was undertaken using non-neurectomized mice or mice whose sciatic and saphenous nerves had been transected or in which not more than a 2-mm segment was removed at the level of the distal end of the femur (Experiment B, Table 5). As previously, the extremity was amputated in the region of the upper femur, 1, 4, 8 or 12 hours after injection of CVS virus, pool 5. Although all 25 non-neurectomized controls died, no deaths occurred in non-neurectomized mice when amputation was performed one or four hours after injection; mortality was 20 of 23 (87%) and 25 of 25 (100%) when amputation was delayed eight and 12 hours, respectively. No neurectomized mice died of rabies when amputation was performed 1, 4, 8 or 12 hours after injection. Mortality in the neurectomized, non-amputated controls was 3 of 25 (12%).

Similar experiments with non-neurectomized mice were also carried out with strain 4727 of mouse encephalomyelitis virus. No saving effect was evident when amputation was performed as early as 5, 15, 30 or 60 minutes after injection (Experiment C, Table 5) or in a second experiment when amputation occurred 1, 4, 24 or 72 hours after injection (Experiment D, Table 5). Mortality in the controls in these two experiments was 6 of 41 (14.6%) and 29 of 42 (69%) respectively.

Fluorescent antibody studies

Attempts were also made to demonstrate virus by fluorescence in the salivary glands, the sciatic nerves proximal to the injected foot-pad and of the opposite limb, the spinal cord, and the brains of groups of mice inoculated in a hind foot-pad with CVS virus, pool 5, or a 10% suspension of strain 61-797F of street virus. Tissues, frozen and sectioned at $-10°C$ to $-12°C$, were obtained from five mice inoculated with each strain of virus 1, 2, 4, 8 and 12 hours and 1, 2, 3, 4 and 5 days or more after injection and at death. Tissues from mice inoculated with street virus were also obtained on the 6th through the 13th days after injection. These were examined by fluorescence, using essentially the method of Goldwasser & Kissling (1958).

In mice inoculated with CVS virus, antigen was regularly first demonstrated in the spinal column at 48 hours, and in the sciatic nerve of the inoculated limb and in the brain simultaneously at 72 hours, and was regularly present in all tissues except the salivary gland in every animal examined after longer intervals, whether the animal was sacrificed or died of rabies. Results with street virus were comparable although the interval between injection and demonstration of virus was somewhat longer. Virus was first demonstrated simultaneously in the sciatic nerve of the injected limb and in the spinal cord at 72 hours and in the brain and sciatic nerve of the opposite limb at 144 hours. Excluding salivary glands, virus was regularly present in all tissues examined 144 or more hours after injection. With both strains, demonstrable antigen in the sciatic nerve was present largely in the form of bead-like chains and increased with time in size and abundance. Accurate determination of the site of virus was not possible by the methods used, although antigen appeared to be located within and at the periphery of nerve fibres. Although not detected in the salivary glands of mice inoculated with CVS virus, virus was detected in the salivary glands of one or more animals inoculated with street virus on the 7th, 12th and 13th days but not on the 8th through 11th days or at death. Virus, when detected, appeared to be in nerve fibres or in glandular cells adjacent to nerve fibres.

Viraemia studies

Since viraemia occurs in mice and rabbits after injection of rabies virus (Wong & Freund, 1951), it was considered a possible mechanism of infection in the occasional neurectomized animal succumbing to rabies. To explore this possibility further, groups of 20, 23 and 72 neurectomized mice weighing 25-30 g were given injections of CVS virus, pool 4, in the foot-pad. One group served as controls; mice in the other two groups were traumatized intracerebrally three and six hours respectively after infection, by inserting into and withdrawing from the brain a 26-gauge needle. Mortality in the traumatized animals was none of 20 and 3 of 23, respectively; the latter was significantly different statistically ($P \simeq 0.013$) from the result (0/72) in non-traumatized mice, suggesting that viraemia occurring six hours after foot-pad inoculation occasionally resulted in death. Mortality in virus controls was 38 of 40 (95%); this is a highly significant difference from the results with traumatized mice with $P \simeq 10^{-13}$ and 10^{-10}, respectively; *a fortiori*, there was a highly significant difference between the controls and non-traumatized neurectomized mice. Similar but less striking results were obtained when

groups of 22-24 neurectomized mice were inoculated intracerebrally with sterile saline 1, 4, 8, 12 and 24 hours after injection of virus in the foot-pad. Mortality was 2, 2, 3, 0 and 1, respectively, as compared with 25 of 25 in the virus controls and 1 of 23 in the neurectomized group not inoculated with saline.

The possibility of blood-borne infection was further investigated by intravenously injecting 13 mice weighing 25-30 g with 0.05 ml of CVS virus, pool 4. Within three minutes all showed signs of severe shock and three died. The surviving mice died of rabies 8-10 days after injection; the median was 9 days. Twenty-two mice given the same dose by the intracaudal or combined intravenous-intracaudal route did not show signs of shock but also died, although both onset of illness and death occurred one to two days later than in animals inoculated by the intravenous route only.

Positive results were also obtained when 10 4-day-old mice were inoculated in the foot-pad with 0.01 ml of CVS virus, pool 5, killed one hour after injection, and the carcass of each animal, minus the injected limb, ground with diluent to yield a 10% suspension, each suspension being injected intracerebrally into eight mice. Virus was recovered from the carcasses of three of the ten mice tested. Since the interval between injection and sacrifice was too short to permit nerve-borne transmission to or above the site of amputation, presumably the presence of virus in the rest of the carcass resulted from blood- or lymph-borne transmission.

Salivary gland tropism

The mechanisms underlying salivary gland infection were initially studied in nine dogs injected bilaterally in the cerebral hemisphere with 0.02 ml of a 1: 80 000 suspension of street virus, strain 61-797F. Intracerebral injection was performed because of the difficulty of obtaining infective salivary glands in dogs when virus is injected peripherally. Prior to injection all the dogs had portions of the right lingual nerve removed and seven had the cranial cervical ganglion on the right side removed or its afferent nerve severed.

Seven dogs succumbed to rabies between 10 and 18 days after injection; the median was 11 days (Table 6). As demonstrated in mice, virus was pre-

TABLE 6

VIRUS CONCENTRATION IN BRAIN AND SALIVARY GLANDS OF DOGS INOCULATED INTRACEREBRALLY AND FOXES INOCULATED INTRAMUSCULARLY WITH RABIES VIRUS, STRAIN 61-797F

Dog. No.	Nerve transected or removed	Interval between challenge and death (days)	Virus titre [a]			Fox No.	Nerve transected or removed	Interval between challenge and death (days)	Virus titre [a]		
			Brain	Left salivary gland	Right salivary gland				Brain	Left salivary gland	Right salivary gland
331	Lingual	18	2.5	3/40	7/48	351	Lingual + cranial and cranial ganglia	20	1.4	0/18	1/20
336	Lingual	11	4.3	2.5	0/9						
333	Lingual + cranial and cranial ganglia	11	4.3	8/8	1/10	352	,,	18	3.3	1/9	1/10
						353	,,	15	3.3	5.5	2.6
334	,,	10	4.9	2/10	0/9	354	,,	16	3.3	4.8	4/19
337	,,	10	3.9	1/10	0/10	355	,,	16	3.6	2.5	2/20
338	,,	13	4.6	4/9	0/10	356	,,	14	3.9	5.0	1/20
339	,,	14	4.9	4.5	1.6	357	,,	19	3.8	2.1	1.4
						358	,,	22	3.1	4.9	1/20
						359	,,	20	3.5	5.1	1.5
						360	,,	23	4.9	5/15	0/19

[a] Virus titre expressed as log to the base 10; or, where the ED_{50} was less than 1.0, as the number of mice dying of rabies over the number inoculated.

sent in the brain and in one or both salivary glands of each dog; brain titres ranged from $10^{2.5}$ to $10^{4.9}$. Except for dog 331, in which only the lingual nerve was cut, virus was present either in the mandibular salivary gland on the non-operated left side only (dogs 334, 336, 337 and 338) or in the neurectomized right gland in reduced amounts (dogs 333 and 339). Titration in mice of suspensions prepared from the left and right glands of dog 339 yielded titres of $10^{4.5}$ and $10^{1.6}$, respectively, indicating that nearly 1000-fold more virus was present in the non-neurectomized gland. Dog 331, a portion of whose lingual nerve only was removed, had the lowest brain titre, $10^{2.5}$, and was apparently the only dog in which more virus was demonstrated in the neurectomized gland.

Efforts were next made to study the mechanisms by which salivary glands become infected under conditions more closely approximating those in the field. Foxes were used since they are more susceptible to rabies than dogs and salivary gland infection usually follows intramuscular injection of strain 61-797F of street virus. Accordingly, 10 foxes, in which the lingual nerves had been transected and the cranial cervical ganglion removed on the right side, were inoculated bilaterally in the masseter muscle with 0.015 ml of a 1: 50 000 suspension of virus. All foxes died of rabies between the 14th and 23rd day; the median was 18.5% (Table 6). The titre of virus in the brain varied from $10^{1.3}$ to $10^{4.9}$. Atrophy of the neurectomized right salivary gland, also observed in the study with dogs, occurred consistently and resulted in a loss of weight varying from 22.9% to 51.9% when compared with the weight of the non-neurectomized gland on the opposite side; the median was 44.4%. Although virus was demonstrated after death in one or both salivary glands of all foxes, there was fivefold to more than 10 000-fold more virus present in the non-neurectomized gland of all foxes except two. Foxes 351 and 352 were exceptions in that virus was demonstrated in trace amounts in the non-operated gland only of the former and in both glands of the latter. More virus was present after death in the non-neurectomized glands than in the brains of foxes 353, 354, 356, 358 and 359. The results in foxes, as in dogs, suggest that salivary glands customarily become infected *via* the peripheral nerves after either intracerebral or intramuscular injection of virus but that other than nerve-borne transmission may play a role of undetermined importance. Blood taken at time of death was not tested for presence of rabies virus.

DISCUSSION

These studies support and extend the observations of Di Vestea & Zagari (1889a, 1889b) that rabies is primarily a nerve-borne disease and also confirm the impressions of Pasteur (1889) and Roux (1889) that blood-borne transmission is possible. Blood-borne infection in nature is probably the exception rather than the rule and is believed less likely in man, in whom resistance to rabies is high, than in animals of certain species known to be highly susceptible, such as the hamster, fox, and cow. Air-borne infection is occasionally possible (Remlinger & Bailly, 1938; Constantine, 1962). It should be emphasized that the virus dose in most experiments was probably greater than that usually encountered in nature except where exposure results in death.

Herpes simplex virus was the only one among the other agents tested that, like rabies, ordinarily moves centripetally *via* the peripheral nerves. These studies thus confirm the unpublished work of Wilday cited by Burnet (1960).

Kligler & Bernkopf (1943), Habel (1941) and Schindler (1961) showed that rabies virus may persist at the site of injection up to 96 hours following injection. The speed with which virus ascends peripheral nerves and the short interval between injection and onset of illness or demonstration of virus in the central nervous system in these studies suggest that multiplication at the site of injection is not necessary to initiate infection. The impression grows that in most instances the outcome is dependent on an infective dose being deposited at the time of exposure. Demonstration of virus at the site of injection after 48 hours at least in animals injected with fixed virus may be influenced by virus present in peripheral nerves after centrifugal spread.

Results with fluorescence generally parallel those of Kligler & Bernkopf (1943) and Schindler (1961), who used mouse-injection tests to demonstrate virus in nerve tissue after intramuscular and foot-pad injection respectively. Either better techniques are required to demonstrate virus in nerve tissue immediately after injection or the agent is present in form or amounts undetectable by fluorescence or mouse-inoculation tests.

The evidence in dogs and foxes inoculated intracerebrally and intramuscularly, respectively, with salivary gland suspensions of street virus unaltered by further passage in animals indicates that prior removal of portions of the lingual nerve and cranial cervical ganglion substantially impedes transmission

of virus to the salivary gland. The source of virus in the neurectomized gland of some animals and the effect of atrophy on virus titre are difficult to assess at this time. Possibilities include failure to remove all afferent nerve fibres, the presence of virus in blood at death, or blood-borne transmission prior to death. Our results in both dogs and foxes are thus less clear-cut than those of Bertarelli (1904) in dogs.

ACKNOWLEDGEMENTS

The authors are grateful to Dr W. R. Thompson for statistical analyses and to Dr George Snook and Mr W. Goodrich for fluorescent microscopy.

RÉSUMÉ

Les expériences effectuées par les auteurs montrent que le virus rabique est généralement transmis du lieu d'inoculation au système nerveux central par voie nerveuse, mais qu'une transmission non nerveuse peut s'effectuer chez les souriceaux et les jeunes rats, dans des espèces très sensibles comme le hamster, ou chez des animaux dont la résistance a été réduite par un traumatisme ou un shock.

Si l'on injecte du virus rabique dans la patte de souris dont de longs segments du nerf sciatique et du nerf saphène ont été au préalable prélevés chirurgicalement, l'on s'aperçoit que la proportion d'animaux mourant de la rage est inférieure, de façon hautement significative, à celle des animaux indemnes ayant reçu une injection identique. Un phénomène analogue s'observe après injection de virus herpétique, mais non si l'on injecte du virus de chorioméningite lymphocytaire ou du virus de l'encéphalomyélite du rat (souches GD7 et FA).

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